measured in the supernatant and washouts using Luminex. ASCs were co-cultured with MACS isolated bone marrow (BM-) PMNs and analyzed using histology, qPCR and Luminex.
Results: Injection of ASCs into day 7 CIOA knee joints (when synovitis is highest) caused a strong influx of immune cells into the joint cavity shortly after injection (6 hours), which had largely disappeared after 24 hours. Immunohistochemistry revealed that particularly PMN-like cells were attracted. Synovial gene expression of neutrophil attracting chemokines KC, CXCL5, and CXCL7 was increased. In line with this, IL-1β stimulated ASCs injected in naive knee joints also resulted in massive influx of PMN-like cells. IL-1β and IFN-γ (as a positive control) stimulation of ASCs *in vitro* strongly enhanced gene expression of KC, CXCL5, and CXCL7 and protein levels of KC. Finally, we co-cultured ASCs with BM-PMNs in the presence of IL-1β or IFNγ. After 3 hours, a clear clustering of neutrophils around ASCs was observed which significantly decreased protein levels of KC (-69% after 24h; -76% after 48h).
Conclusions: ASCs attract PMN-like cells when injected locally into a day 7 CIOA knee joint expressing low levels of IL-1β. *In vitro*, IL-1β stimulated ASCs show an increase in chemokine expression, leading to attraction and clustering with neutrophils and significantly decreased levels of pro-inflammatory factors like KC. The anti-inflammatory effect of locally applied ASCs into OA joints showing synovitis may be triggered by IL-1β and attraction of PMN-like cells.

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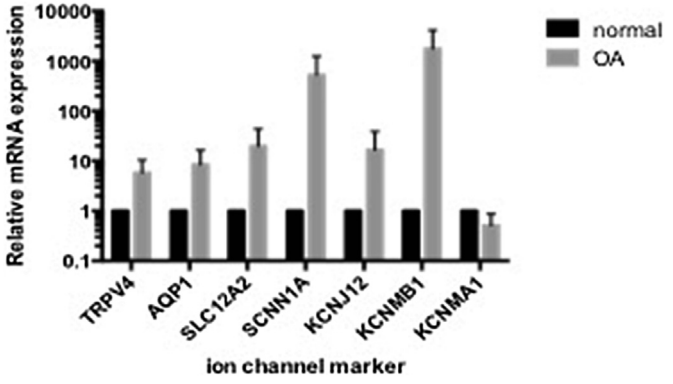
THE CHARACTERIZATION AND FUNCTION OF ION CHANNELS IN SYNOVIAL FLUID DERIVED MESENCHYMAL PROGENITOR CELLS

K.L. Bertram, R.L. Krawetz, U. Banderali. *Univ. of Calgary, Calgary, AB, Canada*

Purpose: In Osteoarthritis (OA) resident synovial fluid mesenchymal progenitor cells (sfMPCs) have greater proliferative ability but reduced chondrogenic capacity. One possible influence on the phenotype of these cells is the physiological environment of the joint. The osmolality of OA joints is significantly lower (~280mOsm) compared to healthy joints (~400mOsm). It was previously demonstrated that changes in osmolality can regulate the expression of chondrocyte gene expression, specifically Sox9. However, it is yet unknown if changes in osmolality regulate the gene expression in sfMPCs, and by extension, chondrogenesis of this cell population. The objective of this study was to determine the response of sfMPCs to changes in environmental osmolality conditions during chondrogenesis and by extension, if differential ion channel expression and function in normal and OA sfMPCs is the mechanism by which regulation of osmolality may occur.
Methods: Synovial fluid samples were collected from normal, OA and RA human knee joints. The osmolality of the fluid was quantified and the derivation/differentiation media was modified to span a range of osmolalities (264-375 mOsm). Chondrogenesis was measured with Alcian blue staining of cultures in addition to quantitative PCR (qPCR) using probes to Sox9, ACAN and Col2A1. Gene expression of six ion channels (TRPV4, SCNN1a, SLC12a2, AQP1, KCNMa1-KCNMb1 complex, KCNJ12) was studied in normal and OA sfMPCs using qPCR and flow cytometry was used to study the protein expression of TRPV4, KCNMb1, and KCNJ12 at the single cell level. A voltage clamp experiment was conducted to evaluate the functional potassium channels in both cell types.
Results: sfMPCs from arthritic joints demonstrated decreased chondrogenic potential compared to sfMPCs isolated from normal synovial fluid. Furthermore, the sfMPCs retained increased chondrogenic potential if differentiated under the same osmolality conditions for which they were initially derived within. Yet, with the change in osmotic environment the cell volume did not change in either case. qPCR results indicated that all ion channel genes examined, except KCNMa1, were upregulated in OA sfMPCs compared to normal.
However, patch clamp results demonstrated that OA sfMPCs showing detectable potassium inward rectifier current (54% vs. 79% in normal) and the amplitude of this current (measured at -80 mV) were significantly reduced compared to normal sfMPCs.
Flow cytometry results showed a significant decrease in TRPV4 in OA sfMPCs compared to normal cells, and normal vs. OA cells demonstrated a differential response to changes in extracellular calcium levels. Interestingly, however, the potassium inward rectifier channel (KCNJ12) and the calcium activated potassium channel (KCNMb1) demonstrated very little change between OA and normal cells.
Conclusions: Synovial fluid osmolality regulates the chondrogenic potential of normal and OA sfMPCs. Ions are regulated by both normal and OA sfMPCs through a combination of ion channels. These results suggest that there is differential ion channel regulation at the functional level, the protein level, and the gene level in OA and normal sfMPCs. Uncovering more about the regulation of ion channel proteins OA sfMPCs may uncover novel pharmaceutical targets for Osteoarthritis treatments in the future.



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HUMAN SYNOVIAL FLUID DERIVED MESENCHYMAL STEM CELLS EXPANDED UNDER LOW OXYGEN CONDITIONS AND IN A SERUM-FREE ENVIRONMENT EXHIBIT ENHANCED LINEAGE-SPECIFIC CHONDROGENIC POTENTIAL

E. Heik †, D.A. Hart ‡, A. Sen †. † *Pharmaceutical Production Res. Facility, Schulich Sch. of Engineering, Univ. of Calgary, Calgary, AB, Canada;* ‡ *McCaig Inst. for Bone and Joint Hlth., Hlth. Sci. Ctr., Univ. of Calgary, Calgary, AB, Canada*

Purpose: Articular cartilage is an avascular tissue with a sparse chondrocyte cell population and has limited capacity for self-repair. The formation of lesions within cartilage, through injury or trauma, can initiate a degenerative process with the end result being osteoarthritis. Mesenchymal stem cells derived from the synovial fluid (SF-MSCs) of articulating joints have the ability to effectively form cartilage, and thus represent a potential candidate cell type for the development of cellular therapies aimed at repairing cartilage lesions. However, they can only be isolated in very small numbers, and thus, must be maintained in carefully controlled culture vessels that provide an environment conducive to rapid cell proliferation in order to generate clinically relevant quantities of cells. It is becoming increasingly apparent that oxygen tension in culture may play a very important role in both stem cell proliferation and differentiation. Moreover, the removal of animal-derived serum from the culture would facilitate the clinical translation of stem cell based therapies. Here the impact of oxygen level on the proliferation and differentiation potential of SF-MSCs under serum free conditions was evaluated.
Methods: Cell populations were isolated (under serum-free conditions) from human synovial fluid (collected from the non-osteoarthritic knee of patients) using established protocols, and characterized to verify the presence of SF-MSCs. Each isolated population was evaluated in a two-factor, two-level factorial manner (normoxic (21%) versus hypoxic (3%) oxygen levels; serum-containing versus serum-free medium). Cell growth kinetics, cell viability, cell morphology, and defining MSC characteristics were assessed for each condition to determine the most optimal for subsequent serial SF-MSC expansion in culture. Cells were grown in each condition and then exposed to either a standard osteogenic or adipogenic lineage protocol, or a chondrogenic differentiation protocol to assess which set of conditions was most conducive for chondrogenesis and subsequently evaluated qualitatively and quantitatively for cartilage characteristics. Statistical significance was evaluated using ANOVA.
Results: SF-MSCs propagated under hypoxic (3% oxygen) conditions, and in a serum-free environment displayed a higher average proliferation rate (doubling time = 21.73 h) compared to cells grown in serum-containing medium and normoxic (21% oxygen) conditions (doubling time = 55.45 h). Moreover, serum-free expanded cells were smaller in size and maintained a more round morphology compared to cells in serum-containing medium, which were bigger, and more

elongated. CFU-F assays carried out under 3% oxygen conditions displayed greater numbers of colonies formed over those grown in 21% oxygen (69% vs. 53%). Osteogenic and adipogenic differentiation of SF-MSCs under 3% oxygen was reduced over cells differentiated in 21% oxygen. Chondrogenic differentiation under low oxygen tension of 3% oxygen resulted in elevated levels of GAG/DNA over cells differentiated in ambient oxygen (mean values of 3.7 μg/μg vs. 1.9 μg/μg).

Conclusions: These studies demonstrate that human SF-MSC populations can be expanded under serum-free conditions without loss of lineage diversity, and that this proliferation can be increased by serially propagating the cells under 3% oxygen tension to enhance chondrogenic potential. The propensity of these cells for chondrogenic differentiation increased when the oxygen tension was lowered from 21% to 3% oxygen, in contrast to the osteogenic and adipogenic lineages. Such low oxygen conditions may better reflect the endogenous oxygen levels in avascular cartilage. Thus, these findings will contribute to the optimization of bioprocesses aimed at scaling-up and differentiating SF-MSCs for use in regenerative therapies for articular cartilage.

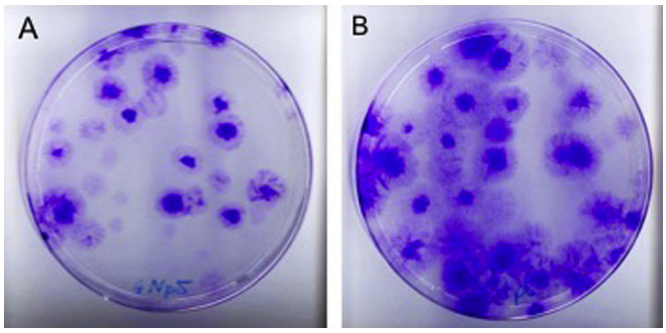

Figure 1. Representative photomicrographs of a CFU-F assay of cells grown under 21% (A) and 3% (B) oxygen, to determine their colony forming ability.

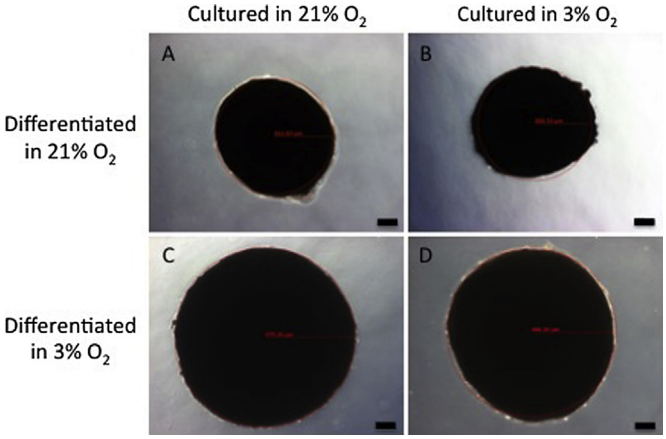

Figure 2. Chondrogenic pellets differentiated under 21% (A,B) and 3% (C,D) oxygen. Scale bars represent 100 μm.

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TGFβ, ACTIVIN A AND NODAL ACTIVATE SMAD2/3 SIGNALING IN MESENCHYMAL STEM CELLS, BUT ONLY TGFβ INITIATES CHONDROGENIC DIFFERENTIATION

L.M. de Kroon †‡, E. N. Blaney Davidson †, R. Narcisi ‡, H.M. van Beuningen †, G.J. van Osch ‡, P.M. van der Kraan †. †*Radboud Univ. Med. Ctr., Nijmegen, Netherlands;* ‡*Erasmus Univ. Med. Ctr., Rotterdam, Netherlands*

Purpose: Cartilage damage is a major problem in osteoarthritis. Application of bone marrow-derived mesenchymal stem cells (BMSCs) to heal damaged cartilage is promising due to their capacity to differentiate into chondrocytes. However, clinical results from BMSC-based therapies are suboptimal because chondrogenic differentiation is variable, and the functional properties of repair tissue differ from native cartilage. To tackle this problem, we need to better understand the molecular events during chondrogenic differentiation of BMSCs. Previously, we showed that the SMAD2/3 pathway is crucial for chondrogenic differentiation of BMSCs. Transforming Growth Factor-β (TGFβ) is well known to activate this pathway by initiating phosphorylation of SMAD2/3 proteins (pSMAD2/3). Besides TGFβ, both Activin A and Nodal are known to induce pSMAD2/3. Therefore, we investigated whether Activin A and/or Nodal, like TGFβ, can initiate chondrogenesis in BMSCs.

Methods: Human fetal BMSCs (purchased from ScienCell) were pellet-cultured in serum-free chondrogenic medium without ligands or with either 10 ng/mL TGFβ1; 10 or 100 ng/mL Activin A; 10 or 100 ng/mL Nodal for 1 or 7 days. At day 1, proteins were isolated from stimulated and unstimulated pellets for western blot analysis of pSMAD2/3. To observe whether cartilage matrix was formed after 7 days, BMSC pellets were harvested for histological evaluation of proteoglycan deposition and mRNA expression analysis of aggrecan, collagen type II and pSMAD2/3-responsive gene plasminogen activator inhibitor-1 (*PAI1*). Relative mRNA levels were measured using Q-RT-PCR and corrected for ribosomal protein S27a expression.

Results: Activin A, TGFβ1, and Nodal dose-dependently induced SMAD2/3 phosphorylation compared to unstimulated BMSC pellets. pSMAD2/3 levels were highest upon TGFβ1 stimulation and lowest upon Nodal. This was also reflected by *PAI1* gene expression. However, despite all three ligands were proven to be bioactive, only TGFβ1 initiated chondrogenic differentiation of fetal BMSC pellets, as shown by increased *ACAN* and *COL2* expression. Moreover, chondrogenesis was confirmed in TGFβ1-stimulated pellets by positive proteoglycan staining, whereas pellets stimulated with Activin A or Nodal were devoid of cartilage-like tissue.

Conclusions: Previously, SMAD2/3 phosphorylation was found crucial for initiating chondrogenesis in BMSCs. In the present study we demonstrate that TGFβ initiated chondrogenic differentiation of BMSCs, whereas Activin A and Nodal did not. Our data suggest that amongst the pSMAD2/3-inducing ligands (Activin A, TGFβ, or Nodal), only TGFβ initiates chondrogenic differentiation of BMSCs. To improve cartilage formation by BMSCs, all molecular events induced by TGFβ in mesenchymal stem cells needs to be further explored.

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CHONDROGENIC PROGENITOR CELLS ARE KEY PLAYERS IN THE REGENERATION OF CARTILAGE TISSUE

N. Miosge. *Univ. Goettingen, Goettingen, Germany*

Purpose: The regeneration of diseased hyaline cartilage continues to be a great challenge, mainly because degeneration overtaxes the tissue's self-renewal capacity.

Methods: Up to date, more than 600 samples of adult osteoarthritic cartilage, without signs of rheumatoid involvement, were obtained from the knee joints of patients (ages: 65 - 75 years) suffering from late-stage osteoarthritis (OA) after total knee replacement. Recently, we also included meniscus specimens from late OA (n = 150), as well as samples from rheumatoid arthritis (n = 40) in our investigation of progenitor cells in situ. Histology, immunohistochemistry and staging was carried out as described. Standard explant cultures were performed. A transcriptome analyses and proteome analysis further characterized the progenitor cell populations. We performed dilution cloning and lentiviral transfection of hTERT of the cells. Quantitative real-time RT-PCR, as well as Western blotting was applied after overexpression and RNA silencing experiments for various chondrogenic mediators, for example, sox9, smad2 or HMGB2. Cell stimulation experiments involved TGFß3, PDGF or BMP6.

Results: Recently, we demonstrated that repair tissue from human articular cartilage during the late stages of osteoarthritis harbors a unique progenitor cell population, termed chondrogenic progenitor cells (CPCs). These exhibit stem cell characteristics such as clonogenicity, multipotency, and migratory activity. The isolated CPCs, which exhibit a high chondrogenic potential, were shown to populate diseased tissue ex vivo. Moreover, down-regulation of the osteogenic transcription factor Runx2 enhanced the expression of the chondrogenic transcription factor Sox9. This, in turn, increased the matrix synthesis potential of the CPCs without altering their migratory capacity (Koelling et al., 2009, Cell Stem Cell). Furthermore, we analyzed sex-specific differences in the regenerative potential of CPCs and the involvement of sex hormones as well as of primary cilia. Migration of CPCs is influenced via PDFG receptors. Here, we describe similar progenitor cell populations in cartilage tissue of patients with rheumatoid arthritis, as well